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Substance Group:	**Group 25 – Succinimide Dispersants**
Summary Prepared by:	**Petroleum Additives Panel** **Health, Environmental & Regulatory Task Group**
Date of last update:	**December 2006**
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1.0 Physico-Chemical Properties

Robust Summary 25 – Water Solubility – 1

CAS No.	67762-72-5
Test Substance Name	2,5-pyrrolidinedione, 1-[2[[-2[[2-[(2-aminoethyl) amino] ethyl] amino]ethyl]amino]ethyl]-monopolyisobutenyl derivitives-
Method/Guideline	Similar to OECD Test Method 105
GLP (Y/N)	Not Specified
Year	Not Specified
Remarks for Test Conditions	The test material was first placed into a hexane washed dialysis bag and subjected to Soxhlet extraction using hexane in order to remove any diluent oil from the test material. The dialysis bag was then opened and the contents washed into glass vials using hexane. The hexane was blown off at 55°C under a nitrogen stream to constant weight. The test material was then loaded onto the generator column packing and water was passed through the column at 0.7 mL/minute. The effluent line from the generator column (0.020"ID Teflon) was connected to a C_{18} absorbent cartridge that would trap any soluble hydrocarbon material. After passage of an appropriate amount of water (approximately 1 L) the cartridge was removed from the system and flushed with nitrogen in order to remove any remaining water. Any absorbed material was then stripped from the cartridge by back flushing the cartridge with 5 mL of methanol, 5 mL of dichloromethane and 6 mL of n-hexane. These flushes were collected in a small glass vial. The content of the vial was then evaporated to dryness under a nitrogen purge at 55 °C. The concentration of the test material was calculated from the nitrogen content of the glass vial by first dissolving the contents in a suitable solvent and analyzing for nitrogen by chemiluminescence using a Dohrmann Analyzer. The analyzer was calibrated using solutions prepared by diluting the de-oiled test material. The nitrogen content of the test material itself was determined by analysis using a Carlo Erba 1500 Nitrogen Analyzer.
Results	The water solubility of the test material was determined to be 0.125 mg/L.
Conclusions	The water solubility of the test material was determined to be 0.125 mg/L.
Data Quality	Reliable with restriction (Klimisch Code). Restriction due to the limited information available regarding analytical results.
References	Rausina, G.A, W. R. Biggs, P. M. Stonebraker and E. A. Crecelius. Using Semipermeable Membrane Devices (SPMDs) to estimate bioconcentration potential of Petroleum Additives. Tribology – Solving Friction and Wear Problems, 10th International Colloquium Tribology, Stuttgart/Qstfildern, Germany. January 9-11, 1996.

Robust Summary 24 – LogKow – 2

<table>
<tr><td><u>Test Substance</u></td><td></td></tr>
<tr><td>CAS #</td><td>67762-72-5</td></tr>
<tr><td>Chemical Name</td><td>2,5-pyrrolidinedione, 1-[2[[-2[[2-[(2-aminoethyl) amino] ethyl] amino]ethyl]amino]ethyl]-monopolyisobutenyl derivitives-</td></tr>
<tr><td><u>Method</u></td><td></td></tr>
<tr><td>Method/Guideline followed</td><td>OECD Guideline for the Testing of Chemicals, 117: Partition Coefficient (n-octanol/water), High Performance Liquid Chromatography (HPLC) Method; TSCA Title 40 of the Code of Federal Regulations, Part 796, Section 1570: Partition Coefficient (n-Octanol/Water) – Estimation by Liquid Chromatography and ASTM E 1147</td></tr>
<tr><td>Test Type</td><td>Partition Coefficient (n-octanol/water), High Performance Liquid Chromatography (HPLC) Method</td></tr>
<tr><td>GLP</td><td>Not Specified</td></tr>
<tr><td>Year (Study Published)</td><td>1996</td></tr>
<tr><td>Log K_{ow} Reference Standards</td><td>Naphthalene
Benzo(a)pyrene
Benzo(c)pyrene
Benzo(gh)pyrene
Coronene</td></tr>
<tr><td>HPLC Conditions</td><td>An octadecyl bonded reverse-phase column and a two-dimensional UV detector were utilized. The test material was dissolved in methyl-t-butyl ether with acetronitrile (20 wt %) added as the mobile phase.</td></tr>
<tr><td>Method Comments</td><td>Several polycyclic aromatic hydrocarbons were used as standards. The retention times of these standards were compared to that of the test substance.</td></tr>
<tr><td>Results</td><td>The mean retention times and Log K_{ow} values of the reference materials and test substance were as follows:

<table>
<tr><td></td><td>Measured Log K_{ow}</td><td>Elution Time</td></tr>
<tr><td>Naphthalene</td><td>3.3</td><td>1.0</td></tr>
<tr><td>Benzo(a)pyrene</td><td>6.0</td><td>1.8</td></tr>
<tr><td>Benzo(c)pyrene</td><td>6.4</td><td>2.0</td></tr>
<tr><td>Benzo(gh)pyrene</td><td>7.0</td><td>4.0</td></tr>
<tr><td>Coronene</td><td>7.6</td><td>4.3</td></tr>
<tr><td>Test Substance</td><td>6.7</td><td>2.4</td></tr>
</table></td></tr>
<tr><td><u>Conclusions</u></td><td>The Log K_{ow} of the test article was 6.7.</td></tr>
<tr><td><u>Data Quality</u></td><td>Reliable without restriction.</td></tr>
<tr><td><u>References</u></td><td>Lubrication Science, 8-2 (1996), pp.145-177.</td></tr>
<tr><td><u>Other</u></td><td>Updated: 12/20/2006</td></tr>
</table>

2.0 Environmental Fate

2.1 Biodegradation

Robust Summary 25-Biodeg-1

Test Substance	
CAS #	84605-20-9
Chemical Name	Amines, polyethylenepoly-, reaction products with succinic anhydride polyisobutenyl derivatives
Remarks	This substance is referred to as Bis alkenyl succinimide derivative in the HERTG's Test Plan for Succinimide Dispersant Category. For more information on the chemical, see Section 2.0 "Chemistry of Succinimide Dispersants" in the HERTG's Final Submission for the Succinimide Dispersant Category.
Method	
Method/Guideline followed	OECD Method 310B; U.S. EPA Method 796.3260; ASTM D5864-95.
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (Study Performed)	1996
Contact time (units)	28 days.
Inoculum	Activated sludge supernatant from domestic wastewater treatment plant and soil filtrate.
Remarks for test conditions	Inoculum: Activated sludge from domestic waste water treatment plant was sieved through a 2 mm screen and adjusted to a target solids level of 2500mg/l by diluting with tap water. Adjusted sludge was aerated and homogenized in a blender. The sludge was allowed to settle for 30 minutes and the supernatant was decanted and added to the flasks at a concentration of 1% v/v. Concentration of test chemical: Sufficient amount of test material was added to each flask, giving 10 mg C/L in the test flasks. Temp of incubation: 23C ± 3C Dosing procedure: No organic solvents were used to facilitate dissolution of the test material. Test material addition was added directly to the treatment group chamber to achieve the final volume. Test Setup: Total volume of liquid in test chambers was 2 L. The biodegradation test was started by bubbling CO_2 free air through the test media. The CO_2 generated within each test chamber was trapped as $BaCO_3$ in the $Ba(OH)_2$ solution and determined by titrating the remaining $Ba(OH)_2$ with 0.05N standardized HCl. Sampling frequency: CO_2 traps were removed for analysis on Days 0, 2, 5, 7, 10, 14, 17, 20, 23, 28 and 29. On day 28, the test was terminated by the acidification of the test chamber to release dissolved CO_2. Controls: Blank and positive controls were included; abiotic and toxicity controls were not. Sodium benzoate was used as the reference

	substance in the positive controls. Analytical method: $Ba(OH)_2$ ("trap") solutions were used downstream of the test flasks to trap generated CO_2 as $BaCO_3$. The CO_2 produced was determined by titrating the remaining $Ba(OH)_2$ with 0.05N standardized HCl. Method of calculating measured concentrations: N/A Other: The sludge was not exposed to the test substance in the laboratory prior to addition to the test flasks. Twenty ml of the supplemented inoculum was combined with test medium within each 4-L erlenmeyer flask. The solutions were aerated with CO2 free air. Standard plate count was 2.1×10^5 CFU/mL.
Results	
Test Substance Degradation, % after time	16 % after 28 days
Kinetic (for sample, positive and negative controls)	Reference (Sodium benzoate): 88%. An average percent biodegradation of 60% was achieved within 5 days, thereby fulfilling the criteria for a valid test reaching the pass level by day 14. Test substance: 16 % (28d)
Breakdown Products (Y/N) If yes describe breakdown products	N
Remarks	
Conclusions	Test substance degraded 16 % in 28 days. The reference substance, sodium benzoate, degraded 88% in the same test period.
Data Quality	(1) Reliable without restrictions.
References	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
Other	Dated : 5-9-02

3.1 Acute Toxicity to Fish

Robust Summary 25-Fish-1

Test Substance	
CAS #	84605-20-9
Chemical Name	Amines, polyethylenepoly-, reaction products with succinic anhydride polyisobutenyl derivatives
Remarks	This substance is referred to as Bis alkenyl succinimide derivative in the HERTG's Test Plan for Succinimide Dispersant Category. For more information on the chemical, see Section 2.0 "Chemistry of Succinimide Dispersants" in the HERTG's Final Submission for the Succinimide Dispersant Category.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guidelines, Section 797.1400 (1992), and OECD Guidelines for Testing of Chemicals, #203, Fish Acute Toxicity Test (1992).
Test Type	Static renewal test
GLP (Y/N)	Y
Year (Study Performed)	1997
Species/Strain	Rainbow trout (*Oncorhynchus mykiss*)
Analytical Monitoring	Total organic carbon (TOC) measurements of initial (0-h) test solutions and after one day of test (24-h) before renewal of test solutions. Water samples were passed through 0.45-micron filter prior to TOC analysis using US EPA Method 415.1.
Exposure Period (unit)	96 hours
Statistical methods	Statistical analysis to determine median lethal concentrations used the binomial/nonlinear interpolation method (Stephan, C.E. 1983. Computer Program for the Calculation of LC50 Values. US EPA, Duluth, MN, USA.; personal communication). Nominal concentrations were used for the calculations.
Remarks field for test conditions (fill as applicable)	Test Organisms: Acquired from Mt. Lassen Trout Farms, Red Bluff, CA, USA; fish were juveniles with an average total length of 46.4 mm and an average wet weight of 1.3 g for the control fish at test termination (no range reported); test loading rate = 0.87 g biomass/L; fish received no pretreatment; fish held for a minimum of 14 days before testing; fish were not fed during the test. Test System: Individual water accommodated fractions (WAFs) were prepared for each test level and renewed daily. A measured weight of test material was added to a measured volume of dilution water in a glass vessel and stirred with for 20 hours. Stirring was accomplished using a Teflon coated magnetic stir bar. The vortex of the WAF was approximately 10%. Following the mixing period, the test solutions were allowed to stand for 4 hour before the water phase was removed. To avoid removing non-soluble test material from the surface, a siphon was used to remove the exposure solutions from the mixing vessels. The siphoned aqueous phase (WAF) was then used in the aquatic toxicity test. About 80% of the solution in each test level was renewed daily after 24, 48, and 72 hours. There were three 15-L replicates per treatment, 10 fish per replicate (30 fish per treatment).

	Dilution Water: Filtered well water collected at Marblehead, MA, USA, and adjusted to a hardness of 40-48 mg/L as $CaCO_3$. The water was passed through activated carbon, a particle filter, an ultraviolet sterilizer, and then stored in a polyethylene tank where it was aerated. Light: 16-hours of light per day using cool-white fluorescent lights with an intensity of 4 μEin/sec/ m^2. Test Temperature: 11.6 to 13.1 C. Water Chemistry: Dissolved oxygen ranged from 5.8 - 9.9 mg/L; pH ranged from 7.0 – 7.7; conductivity ranged from 160 - 180 umhos/cm; alkalinity was not reported. Element: Mortality Test Levels: Control and 1,000 mg/L WAF loading rates.
Results	Nominal concentrations: 96-hour LL_{50} (reported as "LC50" in the report) = > 1,000 mg/L
Remarks	Measured concentration: TOC Loading Level (mg/L) — TOC (range :mg/L) Control — 2.8 - 3.0 1,000 — 3.4 - 4.2 Analytical Monitoring: TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates. Other: • Lethal concentrations are based on nominal loading rates. • Control response was satisfactory. Test Findings: A thin film of insoluble test material was observed in the 1.000 mg/L loading throughout the test in which 97% of organisms survived. No sublethal effects were noted during the test.
Conclusions	96-h LL_{50} = > 1,000 mg/L
Data Quality	(1) Reliable without restriction
References	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
Other	Updated: 5-6-02

Robust Summary #: 25-FISH-2

Test Substance	
CAS #	67762-72-5
Chemical Name	2,5-Pyrrolidinedione,1-[2-[[2-[[2-[(2-aminoethyl)amino]ethyl]amino]ethyl]amino] ethyl]-, monopolyisobutenyl derivatives
Remarks	This substance is referred to as mono alkenyl succinimide derivative in the HERTG's Test Plan for the Succinimide Dispersant Category. For more information on the chemical, see Section 2.0 "Chemistry of Succinimide Dispersants" in the HERTG's Final Submission for the Succinimide Dispersant Category.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1400 (1985/1987/1989), OECD Guideline for Testing of Chemicals #203, Fish Acute Toxicity Test (1984).
Test Type	Static renewal test (Water Accommodated Fraction)
GLP (Y/N)	Y
Year (Study Performed)	1991
Species/Strain	Fathead minnow (*Pimephales promelas*)
Analytical Monitoring	Total organic carbon (TOC) measurements of initial (0-h) test solutions and at 24 hours.
Exposure Period (unit)	96 hours
Statistical methods	Statistical analysis of survival data not warranted because there was no mortality in this study.
Remarks field for test conditions (fill as applicable)	Test Organisms: Acquired from Aquatic Research Organisms, Hampton, New Hampshire, age: juvenile, total length: 38 mm average, wet weight: 0.5 g average (no range reported). Loading: <0.5 g biomass/L, Pretreatment: none, fish held for a minimum of 14 days before testing. No feeding during the test. Test System: Individual test concentrations were prepared by combining the appropriate amount of test substance and dilution water in a glass mixing vessel equipped with a magnetic stirrer and stirring these mixtures for approximately 24 hours, settling the mixtures for approximately 1 hour and siphoning the water phase containing the Water Accommodated Fraction (WAF). Test vessels were 20-liter glass aquaria containing 15 liters of test solution. The solution in each test level was renewed daily after 24, 48, and 72 hours. Two 15-L replicates per treatment, 10 fish per replicate (20 per treatment). Dilution Water: Filtered well water collected at Hampton, New Hampshire and adjusted to the appropriate hardness of 176 mg/L as $CaCO_3$. The water was passed through activated carbon, a particle filter, and then an ultraviolet sterilizer, and then it was stored in a polyethylene tank where it was aerated. Light: 16-h light per day using cool-white fluorescent lights with an intensity of 15 uEin/m^2. Test Temperature: 21.6 to 22.8 C. Water Chemistry: Dissolved oxygen: 6.9 – 8.3 mg/L, pH: 7.0 - 7.8, conductivity: 870 – 900 umhos/cm. Alkalinity not reported.

	Element: Mortality Test Levels: Control, 100, 300, & 1,000 mg/L WAF loading rates. No undissolved test material was seen on the surface of the test vessels during the entire aquatic toxicity test. Test Findings: No mortality or signs of toxicity was observed in all treatments and the control throughout the entire test. Calculation of LL_{50} s: Statistical analysis of survival data not warranted. Analytical Monitoring: TOC levels were between 2.8 - 3.2 mg/L in the control, 3.3 – 4.3 mg/L at 100 mg/L loading, between 3.7 - 4.4 mg/L at 300 mg/L loading and 2.9 – 3.8mg/L at the 1,000 mg/L loading. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates. Reference Substance: No
Results	Nominal concentrations: 96-h LL_{50} >1,000 mg/L. This is equivalent to 96-h LL_0 = 1,000 mg/L (no mortality or toxic signs noted).
Remarks	Measured concentration: n/a Unit: mg/L Statistical results: Statistical analysis of survival data not warranted. Other: • Test results reported in original study as “lethal concentrations” are reported in this summary as “lethal loading”, because test results are based on WAF loading rates. • Control response was satisfactory.
Conclusions	No mortality or signs of toxicity were observed in any of the treatments (100, 300, and 1,000 mg/L WAF loading rates) or in the control throughout the entire test.
Data Quality	(1) Reliable without restriction
References	Ward, T.J. (1993) Acute Toxicity of The Water Accommodated Fractions (WAFs) of CMA 610 to The Fathead Minnow, *Pimephales promelas.* T.R. Wilbury Study #9176-CMA/ESI-610.
Other	Updated: 5/31/02

3.2 Acute Toxicity to Aquatic Invertebrates (e.g. Daphnia)

Robust Summary 25-DAPH-1

Test Substance	
CAS #	84605-20-9
Chemical Name	Amines, polyethylenepoly-, reaction products with succinic anhydride polyisobutenyl derivatives
Remarks	This substance is referred to as Bis alkenyl succinimide derivative in the HERTG's Test Plan for Succinimide Dispersant Category. For more information on the chemical, see Section 2.0 "Chemistry of Succinimide Dispersants" in the HERTG's Final Submission for the Succinimide Dispersant Category.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1300 (1993), OECD Guideline for Testing of Chemicals #202 Daphnia sp. Acute Immobilization Test and Reproduction Test (1984).
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	1996
Species/Strain	Cladoceran, *Daphnia magna*
Analytical Monitoring	Total organic carbon (TOC) measurements were taken of initial (0-h) test solution and at test termination (48-h). Water samples were passed through 0.45 micron filter prior to TOC analysis using US EPA Method 415.1.
Exposure Period (unit)	48 hours
Statistical methods	Statistical analysis to determine median lethal concentrations used the binomial/nonlinear interpolation method (Stephan, C.E. 1983. Computer Program for the Calculation of LC50 Values. US EPA, Duluth, MN, USA; personal communication). Nominal concentrations were used for the calculations.
Remarks field for test conditions (fill as applicable)	Test species: Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture. Daphnids received no pretreatment; no mortality reported in the culture during the 48 hours prior to test start. Test System: Individual water accommodated fractions (WAFs) were prepared for each test level. Each of the five WAFs was prepared by combining the appropriate amount of test substance and dilution water in mixing vessels equipped with a magnetic stir bar and stirred for approximately 20 hrs. Mixing speed was adjusted such that a vortex formed that was approximately 25% of the distance to the bottom of the mixing vessel. Following the mixing period, the test solutions were allowed to stand for 4 hrs before the water phase was removed. The water phase (i.e., WAF) was used for the aquatic toxicity test. Test conditions: Two 300 mL glass beakers that contained 250 mL of test solution were used per treatment. Ten Daphnids were used per replicate (20 per treatment).

	Light: 16 hours light and 8 hours dark per day using cool-white fluorescent lights with an intensity of ~6 uEin/m^2sec. Test temperature: 19.4 – 21.0 ºC Dilution water: Laboratory dilution water with a hardness of 160-180 mg/L as $CaCO_3$. Water chemistry: Dissolved oxygen: 7.4 -8.8 mg/L; pH: 7.4–8.8; conductivity: 560-610 umhos/cm. Element: Immobilization. WAF loading rates: Contol, 130, 220, 360, 600, and 1,000 mg/L WAF loading rates. 10 daphnids per replicate (20 per treatment). No undissolved test material was seen on the surface of the test vessels during the entire test.
Results	Nominal concentrations: 48-hr EC_{50} = > 1,000 mg/L. This is equivalent to 48-hr EL_{50} = > 1,000 mg/L based on WAF loading rates.
Remarks	Measured concentration: TOC Loading Level (mg/L) — TOC (range) Control — 3.7 - 3.8 130 — 4.0 - 3.7 1,000 — 4.2 - 4.3 Analytical Monitoring: TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates. Other: • Effect concentrations are based on nominal loading rates. • Control response was satisfactory.
Conclusions	48-h EL_{50} = > 1,000 mg/L
Data Quality	(1) Reliable without restriction
References	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
Other	Dated: 5-6-02

Robust Summary #: 25-DAPH-2

Test Substance	
CAS #	67762-72-5
Chemical Name	2,5-Pyrrolidinedione,1-[2-[[2-[[2-[(2-aminoethyl)amino]ethyl]amino]ethyl]amino] ethyl]-, monopolyisobutenyl derivatives
Remarks	This substance is referred to as mono alkenyl succinimide derivative in the HERTG's Test Plan for the Succinimide Dispersant Category. For more information on the chemical, see Section 2.0 "Chemistry of Succinimide Dispersants" in the HERTG's Final Submission for the Succinimide Dispersant Category.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1300 (1985, 1987), OECD Guideline for Testing of Chemicals #202 *Daphnia* sp. Acute Immobilization Test and Reproduction Test (1984).
Test Type	Static acute toxicity test (Water Accommodated Fraction)
GLP (Y/N)	Y
Year (Study Performed)	1991
Species/Strain	Cladoceran, *Daphnia magna*
Analytical Monitoring	Total organic carbon (TOC) measurements of initial (0-h) test solutions and at test termination (48-h).
Exposure Period (unit)	48 hours
Statistical methods	Statistical analysis of data not warranted.
Remarks field for test conditions (fill as applicable)	Test species: Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture. Test System: Individual WAFs were prepared for each test level. A measured weight of test material was added to a measured volume of dilution water in a glass vessel and stirred for 24 hours. Stirring was accomplished using a magnetic stir bar. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test. Test conditions: Two 250-mL glass beakers that contained 200 mL of test solution were used per treatment. Light: 16-hour light per day using cool-white fluorescent lights with an intensity of 9 $uEin^{-1}/m^{-2}$. Test temperature: 19.7 – 20.9^{o}C Dilution water: Filtered well water collected at Hampton, New Hampshire and adjusted to the appropriate hardness 176 mg/L as $CaCO_3$. The water was passed through activated carbon, a particle filter, and then an ultraviolet sterilizer and stored in a polyethylene tank where it was aerated. TOC levels were 2.8-3.9 mg/L at the beginning and 1.7-3.2 mg/L at the end of the test.

	Water chemistry: Dissolved oxygen: 7.9 – 9.2 mg/L; pH: 7.0 - 8.4; conductivity: 870 – 900 umhos/cm. Element: Immobilization/mortality Test Levels: Control, 100, 300, & 1,000 mg/L WAF loading rates: 10 daphnids per replicate (20 per treatment). No undissolved test material was seen on the surface of the test vessels during the entire test. Test Findings: No dead organisms were observed in the control or treatment groups at 24 or 48 hours. At 24 hours and 48-hours 100%, and 40% immobilization respectively were reported for the 1,000 mg/L group. Calculation of EL_{50} s: Statistical analysis of survival data not warranted. Exposure period: 48 hours Analytical Monitoring: TOC levels were 2.8 –1.9mg/L in the control, 2.0 - 3.8 mg/l at 100, 2.2 - 3.7 mg/L at 300 mg/L, and 2.2 - 3.0 mg/L at 1,000 mg/L loading. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates.
Results	Nominal concentrations: 48-h EL_{50} >1,000 mg/L. This is equivalent to 48-h EL_0 = 1000 mg/L based on WAF loading rates.
Remarks	Measured concentration: n/a Unit: mg/L Statistical results: Not applicable. Other: • Control response was satisfactory. • Test results are based on WSF loading rates.
Conclusions	The test material was not toxic to daphnids at loading rates tested. The 48 hour median effective concentration was >1000 mg/L (expressed as the nominal concentration used to prepare the WAF) and the 48-hour no observed effect concentration was 1000 mg/L.
Data Quality	(1) Reliable without restriction
References	Ward, T.J. (1993) Acute Toxicity of the Water Accommodated Fractions (WAFs) of CMA #610 to the Daphnid, *Daphnia magna*. T.R. Wilbury Study #9178-CMA/ESI-610.
Other	Updated: 6/4/02

3.3 Acute Toxicity to Aquatic Plants (e.g. algae)

Robust Summary 25-ALG-1

Test Substance	
CAS #	84605-20-9
Chemical Name	Amines, polyethylenepoly-, reaction products with succinic anhydride polyisobutenyl derivatives
Remarks	This substance is referred to as Bis alkenyl succinimide derivative in the HERTG's Test Plan for Succinimide Dispersant Category. For more information on the chemical, see Section 2.0 "Chemistry of Succinimide Dispersants" in the HERTG's Final Submission for the Succinimide Dispersant Category.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guidelines, Section 797.1050, Algal Acute Toxicity Test (1992), and OECD Guidelines for Testing of Chemicals, Method #201, Algal Growth Inhibition Test (1984).
Test Type	Static Test
GLP (Y/N)	Y
Year (Study Performed)	1998
Species/Strain	Freshwater alga, *Pseudokirchneriella subcapitata* formerly called *Selenastrum capricornutum*
Element basis (# of cells/mL)	10^{+4} cells/mL
Exposure period/duration	96 hours
Analytical monitoring	Total organic carbon (TOC) measurements were taken at test initiation (0-hr) and termination (96-hr) from control, low, and high treatment solutions. Water samples were passed through 0.45-micron filter prior to TOC analysis using EPA Method 415.1
Statistical methods	The probit method (Stephan, C.E., 1983. Computer Program for the Calculation of LC50 Values. US EPA, Duluth, MN, USA; personal communication) was used to calculate the EC50 values.
Remarks field for test conditions (fill as applicable)	Test Species: Cells taken from an in-house culture of *Pseudokirchneriella subcapitata* that was originally obtained from the University of Texas at Austin alga collection in January 1997. Test System: Individual water accommodated fractions (WAFs) were prepared for each test level. A measured weight of test material was added to a measured volume of dilution water in a glass vessel and stirred with for approximately 24 hours. Stirring was accomplished using a Teflon coated magnetic stir bar. Mixing speed adjusted such that the vortex extended from the surface approximately 5% of the way to the bottom of the mixing vessel. Following the mixing period, the test solutions were allowed to stand for 4 hour before the water phase was removed. The siphoned aqueous phase (WAF) was then used in the aquatic toxicity test. Test Conditions: A static test was conducted; i.e., there was no daily renewal of test solution. There were three 100-mL replicates per

	treatment loading, each with an inoculum of 10,000 cells/ml. During the test all treatment and control flasks were randomly placed on a rotary shaker adjusted to approximately 100 rpm and located in an incubator during the test under constant light (24 hours/day). Daily cell counts were made visually by means of direct microscopic examination with a hemocytometer. Light: Cool-white fluorescent lights provided a light intensity of approximately 380 footcandles. Test temperature: 23.2 to 24.0 C. Dilution Water: Sterile enriched alga growth media (US EPA, 1978, T.R. Wilbury SOP #6) adjusted to pH 7.5. Measured TOC and total suspended solids in fresh untreated alga media were <1.0 to 1.1 and <10 mg/L, respectively at the beginning of the test and 3.1 to 3.6 and 27, respectively at the end of the test. Test Levels: Control, 33, 65, 130, 250, 500, and 1,000 mg/L WAF loading rates. No undissolved test material was observed in any vessels during the test. Calculation of EL_{50} s : The probit method (Stephan, C.E., 1983) was used to calculate the 72, and 96 hour effective concentrations (EC10s, EC50s, and EC90s). Exposure period: 96 hours
Results	Nominal concentrations: 72- and 96-hour ELgr50 = 320 and 510 mg/L, respectively, based on growth rate measurements. 72- and 96-hour ELb50 = 270 and 370 mg/L, respectively, based on biomass measurements.
Remarks	Measured concentration: TOC Analytical Monitoring: TOC (total organic carbon) levels were <1.0 to 1.1 mg/L and 3.1 to 3.6 mg/L in control vessels at test initiation and at 96 hours, and <1.0 to 1.1mg/L and 1.5 to 1.8mg/L in the 1,000 mg/L test vessels at test initiation and at 96 hours. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates. Unit: mg/L Other: • Effect concentrations based on nominal loading rates. • Test results reported in original study as “effect concentrations” (EC) are reported in this summary as “effective loading” (EL), because test results are based on WAF loading rates and not measured concentrations. • Control response was satisfactory.

Conclusions	No effects on cell size, shape, color, adhesion, or aggregation were noted in any of the loading treatments. The test material was algistatic and not algicidal to the freshwater alga at the highest loading rate tested, 1,000 mg/L. This was determined by removing an aliquot of test media from a 96-hour sample, incubating it in fresh medium, and measuring biomass after 4 days incubation.
Data Quality	(1) Reliable without restriction
References	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
Other	Updated: 5-8-02

Robust Summary #: 25-ALG-2

Test Substance	
CAS #	67762-72-5
Chemical Name	2,5-Pyrrolidinedione,1-[2-[[2-[[2-[(2-aminoethyl)amino]ethyl]amino]ethyl]amino] ethyl]-, monopolyisobutenyl derivatives
Remarks	This substance is referred to as mono alkenyl succinimide derivative in the HERTG's Test Plan for the Succinimide Dispersant Category. For more information on the chemical, see Section 2.0 "Chemistry of Succinimide Dispersants" in the HERTG's Final Submission for the Succinimide Dispersant Category.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1050 (1985, 1987), OECD Guideline for Testing of Chemicals #201 Alga, Growth Inhibition Test (1984).
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	1992
Species/Strain	Freshwater algae, *Pseudokirchneriella subcapitata* formerly called *Selenastrum capricornutum*
Element basis (# of cells/mL)	Approximately 10,000 cells/mL
Exposure period/duration	96 hours
Analytical monitoring	Total organic carbon (TOC) measurements of each test concentration and control test solutions at initiation and at test termination (96-h). Water samples were passed through 0.45-micron filter prior to TOC analysis using EPA Method 415.1
Statistical methods	A parametric one-way analysis of variance (ANOVA) and Dunnett's test were used to calculate the no-observed effect level.
Remarks field for test conditions (fill as applicable)	Test Species: Cells taken from a log-growth phase in-house culture of *Pseudokirchneriella subcapitata* that was originally purchased from University of Texas at Austin alga collection. Several range finding studies were conducted prior to the performance of the definitive studies Test System: This study was conducted twice using WAFs prepared under two different sets of experimental conditions. In the first study Individual WAFs were prepared for each test level. A measured weight of test material was added to a measured volume of dilution water in a glass vessel and stirred for 24 hours. Stirring was accomplished using a magnetic stir bar. Mixing speed was adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end 1-2 inches off the bottom. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test. In the second study Individual WAFs were prepared for each test level. A measured weight of test material was added to a measured volume of dilution water in a glass vessel and stirred for 96 hours

	rather than 24 hours. Stirring was accomplished using a magnetic stir bar. Mixing speed was adjusted such that a vortex formed that extended approximately 5% (rather than 30 to 50%) of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end 1-2 inches off the bottom. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test. At the conclusion of the studies using both the 24 hour WAFs and the 96 hour WAFs, 0.5 ml sub samples of the test media from each flask containing the WAF of the 1000 mg/L solutions of the test substance was combined with 100 ml of fresh media to determine if algicidal or algistatic effects had occurred. These flasks were incubated for 4 days and examined for the presence of algal cells. Test Conditions: Two static tests were conducted using WAFs prepared under two different sets of experimental conditions [24 hrs (30-50% vortex) and 96 hr (5% vortex) WAFs]. There was no daily renewal of test solution. Three 100-mL replicates per treatment, inoculum ~10,000 cells/mL/test. During the tests all treatment and control flasks were randomly placed on an orbital shaker adjusted to approximately 100 cycles per minute under constant light (24 hours/day). Daily cell counts were made visually by means of direct microscopic examination with a hemocytometer. Light: Cool-white fluorescent lights provided a light intensity of 50 uEin/m^2sec 24-h per day. Test temperature: 24 hr WAF Test (30-50% vortex): 23.6 to 24.7^oC 96 hr WAF Test (5% vortex): 23.6-24.7 ^oC Dilution Water: Sterile enriched alga growth media (US EPA, 1978, T.R. Wilbury SOP #6) adjusted to pH 7.5. Measured TOC and total suspended solids in fresh untreated alga media were <1.0 and <10 mg/L, respectively. Test media pH was 6.8 – 7.6 at 0-hour and 7.6 - 10.1 after 96 hours. Test Levels: Control, 1.0, 10, 100, and 1000 mg/L WAF loading rates. During the test with the 24 hrs (30-50% vortex) WAFs, insoluble material was observed on the walls of the 100 and 1000 mg/L WAF test vessel at 24, 48, 72 and 96 hours. During the test with the 96 hr (5% vortex) WAFs, media at all concentration levels were slightly turbid at 24, 48, 72 and 96 hours. All test containers had insoluble material present on the test chamber walls at 24, 48, 72 and 96 hours. Calculation of EL_{50}s and NOELs: Binomial nonlinear interpolation methods (Stephan, 1983) were used to calculate EC_{50}s (i.e., EL_{50}s). A parametric one-way analysis of variance (ANOVA) and Dunnett's test

	were used to calculate the no-observed effect level at 72 and 96 hrs. Method of calculating mean measured concentrations: not applicable. Exposure period: 96 hours Analytical monitoring: 24 hr WAF Test (30-50% vortex): At the beginning of the test, TOC measurements were < 1 mg/L in the control and all treated groups, at the end of the test TOC levels were <1 – 1 mg/L in the control and 1 mg/L WAF; and <1 mg/L in all other groups. 96 hr WAF Test (5% vortex): At the beginning of the test, TOC measurements were generally < 1 mg/L in the control, 1, 10, 100 and 1000 mg/L WAF groups. At the end of the test TOC levels were <1 – 1 in control; 1-4 mg/L in the 1 mg/L WAF, 2 mg/L at 10 mg/L WAF, 2-4 mg/L at 100 mg/L WAF and 1-2 mg/l at 1000 mg/L WAF. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates.
Results	24 hr WAF (30-50% vortex): Based on cell density: 72-hour (mg/L) 96-hour (mg/L) EL_{50} 14 21 NOEL 1.0 1.0 Based on growth rate: EL_{50} 24 34 NOEL 1.0 10 96 hr WAF (5% vortex): Based on cell density: 72-hour (mg/L) 96-hour (mg/L) EL_{50} >1000 >1000 NOEL 10 10 Based on growth rate: EL_{50} >1000 >1000 NOEL 10 10 Regrowth of inhibited cultures from both 1000 mg/L test levels [24 hr WAF (30-50% vortex) and 96 hr WAF (5% vortex)] confirmed that the test material was algistatic.
Remarks	Measured concentration: n/a Unit: mg/L Other: • Test results reported in original study as “effect concentrations” and “no observed effect concentrations” are reported in this summary as “effect loading” and “no observed

	effect levels", respectively, because test results are based on WAF loading rates. • Control response was satisfactory.
Conclusions	At a loading rate of 1000 mg/L the test material was considered algistatic. The 24 hr WAF (30-50% vortex) had an EL_{50} (72-96 hrs) of 14-34 mg/L and an NOEL of between 1 and 10 mg/L (72-96 hrs). The 96 hr WAF (5% vortex) had an EL_{50} (72-96 hrs) of >1000 mg/L and an NOEL of 10 mg/L (72-96 hrs).
Data Quality	(1) Reliable without restriction
References	Magazu, J.P., (1994) Acute Toxicity of the Water Accommodated Fractions (WAFs) of CMA #610 to the Freshwater Alga, *Selenastrum capricornutum.* T.R. Wilbury Study #73-CM-610. Stephan, C.E. (1983). Computer Program for the Calculation of LC50 Values. U.S. EPA. Duluth, MN. Personal Communication.
Other	Updated: 6/4/02

4.1 Acute Toxicity

4.1.1 Acute Oral Toxicity

Robust Summary #: 25-Acute Oral-1

Test Substance	
CAS #	67762-72-5
Chemical Name	2,5-Pyrrolidinedione,1-[2-[[2-[[2-[(2-aminoethyl)amino]ethyl]amino]ethyl]amino] ethyl]-, monopolyisobutenyl derivatives
Remarks	This substance is referred to as mono alkenyl succinimide derivative in the HERTG's Test Plan for the Succinimide Dispersant Category. For more information on the chemical, see Section 2.0 "Chemistry of Succinimide Dispersants" in the HERTG's Final Submission for the Succinimide Dispersant Category.
Method	
Method/Guideline followed	OECD Guideline 401
Test Type	Acute oral toxicity
GLP (Y/N)	Y
Year (Study Performed)	1985
Species/Strain	Rats/Sprague-Dawley
Sex	Male/Female
No. of animals/dose	5 /sex/group
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	0 and 5000 mg/kg
Dose volume	Not Provided
Control group	Yes
Chemical analysis of dosing solution	No
Remarks field for test conditions	A single dose of the undiluted test material was administered intragastrically to five fasted male and female rats. A control group was included. The animals were observed for signs of toxicity or behavioral changes twice daily. Individual weights were recorded on the day of dosing, on days 2 and 7 and at termination. Body weights of treated animals were compared to control using the Student's t-test. All animals were euthanized, and gross necropsies were performed, at the conclusion of the observation period.
Results	LD50 > 5 g/kg
Remarks	There were no deaths during the study. Diarrhea was observed in two treated males and two treated females on the day of dosing and on the following day. There were no significant differences in mean body weight between the treated and control groups. There were no significant necropsy findings evident in the surviving animals.

Conclusions	The test article, when administered to 5 male and 5 female rats had an acute oral LD50 of > 5 g/kg. No significant toxicity was observed.
Data Quality	Reliable without restriction (Klimisch Code).
References	Unpublished confidential business information
Other	Updated: 8/3/01

Robust Summary #: 25-Acute Oral-2

Test Substance	
CAS #	84605-20-9
Chemical Name	Amines, polyethylenepoly-, reaction products with succinic anhydride polyisobutenyl derivatives
Remarks	This substance is referred to as Bis alkenyl succinimide derivative in the HERTG's Test Plan for Succinimide Dispersant Category. For more information on the chemical, see Section 2.0 "Chemistry of Succinimide Dispersants" in the HERTG's Final Submission for the Succinimide Dispersant Category.
Method	
Method/Guideline followed	OECD Guideline 401
Test Type	Acute oral toxicity
GLP (Y/N)	Y
Year (Study Performed)	1996
Species/Strain	Rats/Crl:CD®(SD)BR
Sex	Male/Female
No. of animals/dose	5 /sex/group
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	5000 mg/kg
Dose volume	5.68 ml/kg of body weight (based on an average bulk density of 0.88 g/ml)
Control group	No
Chemical analysis of dosing solution	No
Remarks field for test conditions	A single dose of the undiluted test material was administered intragastrically to five fasted (17-20 hours) male and female rats. A control group was not included. The animals were observed for signs of toxicity or behavioral changes at 1, 2.5 and 4 hours after dosing and daily thereafter. Mortality checks were conducted twice a day for 13 days and on the morning of day 14. Individual body weights were recorded on the day of dosing, on day 7 and at termination. All animals were euthanized at the conclusion of the observation period. Abbreviated gross necropsy examinations were performed on all animals.
Results	LD50 > 5 g/kg
Remarks	There were no deaths during the study. Dark staining of the anal area was observed in three males on days 1 through 5. All animals were normal by day 6 post dosing. Body weight data were unremarkable. There were no visible lesions observed in any of the animals at necropsy.
Conclusions	The test article, when administered to 5 male and 5 female rats, had an acute oral LD50 of > 5 g/kg. No significant toxicity was observed.
Data Quality	Reliable without restriction (Klimisch Code).
References	Unpublished confidential business information
Other	Updated: 8/3/01

4.1.2 Acute Dermal Toxicity

Robust Summary #: 25-Acute Dermal-1

Test Substance	
CAS #	84605-20-9
Chemical Name	Amines, polyethylenepoly-, reaction products with succinic anhydride polyisobutenyl derivatives
Remarks	This substance is referred to as Bis alkenyl succinimide derivative in the HERTG's Test Plan for Succinimide Dispersant Category. For more information on the chemical, see Section 2.0 "Chemistry of Succinimide Dispersants" in the HERTG's Final Submission for the Succinimide Dispersant Category.
Method	
Method/Guideline followed	OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	Y
Year (Study Performed)	1996
Species/Strain	Rats/Crl:CD®(SD)BR
Sex	Male and female
No. of animals/sex	5
Vehicle	None
Route of administration	Dermal
Dose level	2 g/kg (0.014 g/cm^2)
Application area	Approximately 36 cm^2
Control group included	No
Remarks field for test conditions	Prior to the initiation of dosing the back and flanks of each animal were clipped of hair to expose 20% of the total body surface. Animals were reclipped as needed. A single dose of 2 g/kg of the undiluted test material was administered dermally to five male and female rats. The test material was kept in contact with the skin for a period of 24 consecutive hours, on approximately 20% of the total body surface under a semi-occlusive bandage that was loosely over wrapped with a sheet of perforated plastic film. At the end of the 24-hour exposure period, the application site was wiped clean of residual test material with mineral oil, followed by liquid Ivory soap mixed with warm tap water, rinsed with tap water, and dried with a paper towel. The animals were observed for abnormal clinical signs at 1, 2.5, and 4 hours after dosing and daily for the 14-day study period. Dermal examinations were performed 30 minutes post test material removal and on days 3, 7, 10 and 14 according to the Draize method. Individual body weights were recorded on day 1, prior to dosing, and on days 7 and 14. The surviving animals were euthanized at the conclusion of the observation period. Gross necropsies were performed on all animals.
Results	LD50 > 2.0 g/kg (males and females)
Remarks	No mortality was observed. Clinical observations were unremarkable. All animals exhibited body weight gains during the study. No dermal findings were observed at the test material application site. There were no macroscopic findings associated with treatment.

Conclusions	The test article, when administered dermally as received to 5 male and 5 female Sprague-Dawley rats, had an acute dermal LD50 of greater than 2.0 g/kg. No toxicity was observed.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 8/3/01

Robust Summary #: 25-Acute Dermal-2

Test Substance	
CAS #	67762-72-5
Chemical Name	2,5-Pyrrolidinedione,1-[2-[[2-[[2-[(2-aminoethyl)amino]ethyl]amino]ethyl]amino] ethyl]-, monopolyisobutenyl derivatives
Remarks	This substance is referred to as mono alkenyl succinimide derivative in the HERTG's Test Plan for the Succinimide Dispersant Category. For more information on the chemical, see Section 2.0 "Chemistry of Succinimide Dispersants" in the HERTG's Final Submission for the Succinimide Dispersant Category.
Method	
Method/Guideline followed	OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	Y
Year (Study Performed)	1986
Species/Strain	Rabbits/New Zealand White
Sex	Male and female
No. of animals/sex	5
Vehicle	None
Route of administration	Dermal
Dose level	0 and 5 g/kg
Control group included	Yes
Remarks field for test conditions	On the day prior to the initiation of dosing the trunk of each animal was clipped of hair. Animals were reclipped as needed. A single dose of 5 g/kg of the undiluted test material was administered dermally to five male and female rabbits. The test material was kept in contact with the skin for a period of 24 consecutive hours under a plastic sheet that was over wrapped with paper towels to prevent tearing. At the end of the 24-hour exposure period, the application site was wiped clean of residual test material with mineral oil. Collars were placed on all control and treated animals for 24 hours to prevent oral ingestion of residual test material. The animals were observed for abnormal clinical signs frequently after dosing and twice daily for the 14-day study period (once daily on weekends). Dermal examinations were performed on days 1, 7 and 14 according to the modified Draize method. Individual body weights were recorded on day 1, prior to dosing, and on day 2, 7, and 14. The surviving animals were euthanized at the conclusion of the observation period. Gross necropsies were performed on all animals. Sections of skin and any abnormal tissues were examined microscopically.
Results	LD50 > 5.0 g/kg (males and females)
Remarks	No mortality was observed. Slight to well-defined erythema was observed in the treated skin of males and females between days 1 and 7. Slight erythema was observed in the treated skin of all animals one hour after exposure to 5 g/kg of the test material. Three treated females still had well defined erythema six days later, but all had normal skin by day 14. Dermal postulates or abscesses developed in two treated males and one control female. These lesions were observed in the treated area and on the lip between days 7 and 14. Reddened depilitated or flaky skin was observed at previously

	abscessed sites at necropsy on Day 14. The dermal lesions observed in treated males appeared histologically as trace or moderate hyperkeratosis, mild dermatitis and mild acanthosis. The skin of all treated females was histologically normal. The mean body weight of the treated females was 5% lower than control at study termination.
Conclusions	The test article, when administered dermally as received to 5 male and 5 female Sprague-Dawley rats, had an acute dermal LD50 of greater than 5.0 g/kg. Significant dermal findings were observed in the treated males and females during the first week of study and in the males only at study termination.
Data Quality	Reliable with restriction (Klimisch Code). Restriction due to the presentation of only summary dermal findings in the final report.
References	Unpublished confidential business information
Other	Updated: 8/3/01

4.2 Repeated Dose Toxicity

Robust Summary #: 25- Repeated Tox-1

Test Substance	
CAS #	67762-72-5
Chemical Name	2,5-Pyrrolidinedione,1-[2-[[2-[[2-[(2-aminoethyl)amino]ethyl]amino]ethyl]amino] ethyl]-, monopolyisobutenyl derivatives
Remarks	This substance is referred to as mono alkenyl succinimide derivative in the HERTG's Test Plan for the Succinimide Dispersant Category. For more information on the chemical, see Section 2.0 "Chemistry of Succinimide Dispersants" in the HERTG's Final Submission for the Succinimide Dispersant Category.
Method	
Method/Guideline followed	OECD 410
Test Type	28-day dermal toxicity study in rats
GLP (Y/N)	Y
Year (Study Performed)	1985
Species	Rat
Strain	Sprague-Dawley CD, 10-11 weeks of age at initiation of treatment
Route of administration	Dermal, 6 hour/day, to the clipped, unabraided, dorsal surface.
Duration of test	28 days of treatment (Monday through Friday; 28 total doses administered/animal)
Doses/concentration levels	0, 10, 40, and 80% in mineral oil
Vehicle	Mineral Oil
Sex	Males and females
Dose volume	1 ml/kg
Frequency of treatment	Once/day, 5 days/week
Control and treatment groups	12 animals/sex/group. The control animals were administered the vehicle.
Post exposure recovery period	None
Dose range finding study	Yes
Statistical methods	Body weight, body weight gains, food consumption, hematology and clinical chemistry parameters, organ weights and organ/body weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Tests included parametric ANOVA with a Dunnett's test.
Remarks field for test conditions	The test material was applied to the clipped, unabraided dorsal surface of the rats for 6 hours/day, 5 days/week for 28 days. On each dosing day the appropriate dosing suspension was applied to either the anterior or posterior half of the shaved area. Application sites were alternated on each day of dosing. Animal fur was reclipped twice/week. A plastic collar was placed around each animal's neck to prevent ingestion of the test material. Following the 6-hour exposure the collars were removed and the application site was wiped with gauze moistened with mineral oil. (OECD Guideline 410 suggests the use of a gauze patch over the treatment site secured to the trunk with non-irritating tape and wrapped with an elastic sleeve. This procedure was not used during this study. This is considered a minor deviation

	from the suggested Guideline.) Animals were examined for viability and signs of toxicity daily. Detailed clinical observations were made weekly. Pupil response was evaluated pretest, on the first and last day of treatment and weekly during the study. Dermal responses were evaluated (Draize) on the first and last day of treatment and weekly, on Fridays, during the study. Body weights were recorded twice weekly during the study. Food consumption was recorded weekly during the study. Hematology and clinical chemistry parameters were evaluated for 10 animals/sex/group at termination. Macroscopic examinations were performed on all animals. Select organs were weighed. The lungs, liver, spleen, brain, kidneys, testes, ovaries, skin (treated and untreated) and gross lesions were examined microscopically.
Results	
Remarks	No deaths occurred and no compound related signs of toxicity were observed during the study. The physical observations that were observed included ocular and nasal discharges and alopecia on the forepaws and scabs and sores on the neck. These findings were attributed to the use of collars during treatment and were not considered compound related. Normal pupil responses were observed in all animals throughout the study. Slight to well defined erythema with no or slight edema was seen in both sexes of the treated and control groups. No dose related trends were evident in the incidence or severity of skin irritation at any evaluation interval. These findings were attributed to a vehicle effect. Dry, flaky, and/or abraded skin was observed sporadically in all but the high dose group and was not dose related. Body weights and body weight gain were unremarkable during the study. The mean food consumption value of the mid dose males was slightly (statistically significantly) elevated compared to control during the second week of study. This difference actually reflected a slight decrease in control food consumption and was not attributed to exposure to the test material. Food consumption data was unremarkable in all groups at the remaining evaluation intervals. There were no treatment-related differences from control observed in the hematology data of the treated animals following the treatment period. Serum chemistry values were considered unremarkable in the treated animals at termination. There were several, non-treatment related, statistically significant differences observed in several serum chemistry parameters. These included decreases in mean glucose (mid-dose males) and sodium (males), increased direct billirubin (high-dose males) and uric acid in the low dose females. No dose related trends were evident in these data and all of these findings were within the range of corresponding historical control data. These differences were not considered treatment related. There were no alterations in organ weights that were attributed to treatment with the test material. The mean brain weight of the low dose males was slightly (statistically significantly) lower than control.

	However, the corresponding brain to body weight ratios were comparable indicating that the decrease observed in mean absolute weights was not treatment related. Several gross pathological observations appeared sporadically and exhibited no dose related trends. They were not considered treatment related. Findings included red, thickened or scabbed skin in four control males, dilated renal pelvis in three low dose males, one mid dose female and one high dose female, a fluid filled kidney in one low dose male, red salivary nodes in one control female and mottling or multiple red/purple foci on the thymus in one control and one mid dose male. Red foci in the lungs were seen in one mid dose male and a diaphragmatic hernia was noted in one mid dose female. These findings were not considered treatment related. Microscopic examination of treated skin sites showed acanthosis in both high dose and control animals. Necrosis and ulceration of treated skin was observed only in the controls. There was no increase in the incidence or severity of skin lesions in the high dose animals compared to the controls. The observed dermal changes were attributed to exposure to the vehicle and not to treatment with the test material. Other histopathological changes that were observed were spontaneous or naturally occurring lesions in rats of this strain.
Conclusions	The Study Director concluded that the repeated dermal application of this test material caused no observable signs of toxicity. The No Observed Effect Level was 80% in mineral oil.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 8/6/00

Robust Summary #: 25-Repeated Tox-2

Test Substance	
CAS #	84605-20-9
Chemical Name	Amines, polyethylenepoly-, reaction products with succinic anhydride polyisobutenyl derivatives
Remarks	This substance is referred to as Bis alkenyl succinimide derivative in the HERTG's Test Plan for Succinimide Dispersant Category. For more information on the chemical, see Section 2.0 "Chemistry of Succinimide Dispersants" in the HERTG's Final Submission for the Succinimide Dispersant Category.
Method	
Method/Guideline	OECD Guidelines 421 and 407, EPA Guideline 81-8, 82-7, 83-1
Test Type	Combined four week repeated dose oral toxicity, reproduction and neurotoxicity screen in rats
GLP (Y/N)	Y
Year (Study Performed)	1997
Species	Rat
Strain	Sprague-Dawley CD, 51 days of age at initiation of treatment
Route of administration	Orally by gastric intubation
Duration of test	28 Day toxicity phase: 29 or 30 days Reproduction phase: F0 males: 29 day premating period plus mating and postmating periods (70 days total). F0 female: 29 day premating period through day 4 of lactation (54-68 days total). Neurotoxicity phase: 29 days
Dose levels	28 Day toxicity phase: 0, 100, 500 and 1000 mg/kg/day Reproduction phase: 0, 100, 500 and 1000 mg/kg/day Neurotoxicity phase: 0, 1000 mg/kg/day
Vehicle control	Corn Oil
Dose volume	5 mL/kg
Sex	Males and females
Frequency of treatment	Once/day, 7 days/week
Analytical confirmation of concentration.	Homogeneity, stability and weekly dose concentration confirmation.
Control and treatment groups	28-Day toxicity phase: 6/sex/group in all groups plus 6/sex recovery in control and high dose groups. Reproduction phase: 12/sex/group Neurotoxicity phase: 6/sex/group in the control and high dose plus 6/sex recovery in control and high dose groups.
Post exposure recovery period	28 Day toxicity phase: 2 weeks Neurotoxicity phase: 2 weeks
Mating ratio	One male to one female
Duration of mating period	Up to 9 days with initial male; if positive evidence of mating not present (sperm or copulatory plug) then female paired with a second proven breeder male from the same dose group for up to six additional days.
Statistical methods	Anova, Bartlett's test, Dunnett's, Kruskal-Wallis, Dunn's Ranked Sum, Jonckheere's test; F test, Welch's test, t-test, Fisher's Exact Test with Bonferonni correction.
Dose range finding study	3/sex/group at dose levels of 0, 100, 500 and 1000 mg/kg/day treated for 7 days.

Remarks field for test conditions	*Range-Finding, 28 Day Toxicity, Neurotoxicity and Reproduction Study* Viability and Toxicity: twice daily, at least 4 hours apart. Clinical Observations: pretest and weekly throughout the study. Body Weights: pretest, day 1 of treatment, weekly through termination. F0 males: pretest, weekly during premating, mating and post mating periods and at termination. F0 females: pretest, weekly during premating and mating; gestation days 0, 7, 14 and 20; females with litters weighed on lactation days 0 and 4. Unmated females were weighed weekly. Food Consumption: Pretest and weekly during treatment and recovery periods. F0 males: pretest, weekly throughout the study except during mating. F0 females: pretest, weekly during premating, days 0-7, 7-14 and 14-20 of gestation and days 0-4 of lactation for females with litters. Necropsy: 3/sex/group. Macroscopic Examinations: Macroscopic examinations performed on all animals. 28 Day Toxicity Study Phase Hematology, Clinical Chemistry and Urinalysis: 6/sex/group all groups at termination of treatment, 6/sex from the control and high dose group at completion of the two-week recovery period. **Necropsy: Treatment termination-6/sex/group, macroscopic examination. Recovery termination-6/sex/Control and High Dose, macroscopic examination.** Macroscopic Examinations: Macroscopic examinations performed on all animals. Selected organs weighed for all animals. A range of tissues was preserved for all animals in the control and high dose groups. Microscopic Examinations: Gross lesions and tissue masses were examined microscopically for all animals. Neurotoxicity Study Phase Functional Observational Battery (FOB): 12/sex from the control and high dose group pretest and on days 14 and 29 and 6/sex from the control and high dose on day 42. All animals were tested prior to dosing. Animal groups were blind to the evaluator. The FOB included an evaluation of posture, vocalization and palpebral closure, reactivity to general stimuli, assessment of signs of autonomic function including lacrimation, salivation, fur appearance, deposits around the eyes, arousal level and gait, urination and defecation counts, convulsions, tremors, abnormal movements or behaviors, excessive or repetitive actions, piloerection and exophthalmos, approach response, auditory stimuli, tail pinch response, papillary function, hindlimb extensor strength, grip resistance, landing foot splay, air righting ability and body weight. Necropsy: Treatment termination-6/sex/Control and High Dose, macroscopic examination (Day 30). Recovery termination-6/sex/Control and High Dose macroscopic examination (Day 46). Macroscopic Examinations: All males in the control and high dose were perfused for better fixation of the CNS and peripheral nervous tissues. Brain weight and size recorded. The brain and spinal cord

	were sampled for neuropathological evaluation. Tissues sampled included: brain (forebrain, center of cerebrum, midbrain, cerebellum, pons, medulla oblongata), spinal cord (at cervical swellings C3-C7 and at lumbar swellings T13-L4), Gasserian ganglion, Lumbar dorsal route ganglion (T13-L4), Lumbar dorsal root fibers (T13-L4), Lumbar ventral root fibers (T13-L4), Cervical dorsal route ganglion (C3-C7), Cervical dorsal root fibers (C3-C7), Cervical ventral root fibers (C3-C7), Sciatic nerve (mid thigh region), Sciatic nerve (at sciatic notch), Sural, Tibial, Peroneal nerves, Optic nerve and eyes. Microscopic Examinations: The tissues outlined above and all gross lesions and tissue masses were examined microscopically for all neurotoxicity animals. Reproductive Study Phase Pup/Litter Examinations: Litters observed as soon as possible after delivery for number of live and dead pups and pup abnormalities. Thereafter litters observed twice daily for dead pups and/or obvious irregularities through day 4 of lactation. Litter Size: Number of live and dead pups recorded on days 0 and 4 of lactation. Individual Pup Body Weights: Pup weights recorded on days 0 and 4 of lactation. Pup Sex Distribution: Number of male and female pups in each litter recorded on days 0 and 4 of lactation. Necropsy: F0 males Day 71(2 days after the last litter reached lactation Day 4); F0 females with litters sacrificed on lactation Day 4; Unmated females sacrificed 25 days after completion of mating period; Mated females that did not deliver sacrificed 25 days after completion of mating period; F1 pups were sacrificed on lactation Day 4. Macroscopic Examinations: Macroscopic examinations performed on all F0 animals. F1 pups were examined externally and internally including internal sex verification. Tissues retained for possible skeletal evaluation. Selected organs weighed for all animals. A range of tissues was preserved for all animals in the control and high dose groups. Microscopic Examinations: Select tissues examined microscopically for all control and high dose F0 animals. Gross lesions and tissue masses were examined microscopically for all F0 animals.

Results	*Dose Range-Finding Study* No effects of the test material were observed during the dose range finding study on animal survival, clinical observations, body weight and body weight change, food consumption and feed efficiency or postmortem data. The No Observed Effect Level for the dose range-finding study was 1000 mg/kg/day. ***28 Day Toxicity and Neurotoxicity Study Phases*** All animals survived to their scheduled sacrifice. Clinical observations were unremarkable. Body weight and body weight change data were unremarkable in all groups during treatment and recovery. Food consumption data at 100 and 500 mg/kg/day were comparable to control during treatment and recovery. In the 1000 mg/kg/day group food consumption was significantly higher than control during treatment and continued to be elevated in the females during recovery. Feed efficiency in this group was generally comparable to control; however values were lower than control in the 1000 mg/kg/day males at week 4 and during the first week of recovery in the females. Due to the lack of a consistent trend in these data the observed differences from control were not considered by the Study Director, or by this reviewer, to be of toxicological significance. No effects of treatment were observed in the functional observational battery data of the 1000 mg/kg/day group. Landing foot splay distances in the 1000 mg/kg/day group were slightly shorter than control during treatment and recovery. However these data were similar to pretest values. These values did not change during treatment. Neurological insults are usually associated with a lengthening of this distance. This finding was not considered toxicologically significant or treatment related. No treatment related effects were evident in the hematology, clinical chemistry, urinalysis, organ weight, brain size or macroscopic data of the treated animals in the 28-day toxicity or neurotoxicity study phases. The microscopic examination of gross lesions did not indicate any treatment related microscopic findings in the 28-day toxicity study phase. In the neurotoxicity study phase all tissues were within normal limits when examined by light microscopy. The No Observed Effect Level for the 28-day toxicity and neurotoxicity study phases was 1000 mg/kg/day. *Reproductive Study Phase* *F0 (Parental Generation)* All control and treated animals survived to their scheduled sacrifice. Clinical findings were unremarkable. Mean body weight, body weight change, food consumption and feed efficiency were generally unremarkable during the premating period. In some instances body weight gain and food consumption values were slightly elevated compared to control. These differences from control were not considered toxicologically significant. Mean male body weights of the treated animals were generally considered comparable to control during mating and post mating. Some mid and high dose animals

exhibited slight weight loss during the last two weeks of the study. The cause of this finding was unclear. However the lack of any body weight effects during the first eight weeks of study and the lack of any effect on body weight gain suggests that this finding was not treatment related. Postmating period food consumption for the treated males was higher than control (frequently statistically significantly) over the postmating period. This increase in food consumption was not considered an adverse effect or toxicologically significant. Feed efficiency was reduced in the 500 and 1000 mg/kg/day males during the last two weeks of study. These decreases coincided with the reduced body weights observed in some animals in these groups.

Female mating indices for the treated groups were comparable to control. Male mating indices were comparable to control (100%) at 100 (100%) and 500 (100%) mg/kg/day and slightly lower than control at 1000 mg/kg/day (83.3%, 10/12). The lower value in the high dose group was not statistically different from control and was within the range of recent historical control data for the laboratory. It was not considered treatment related.

Mean maternal body weights during gestation were unaffected by treatment. Body weight gain over days 7-14 of gestation in the mid and high dose groups was significantly higher than control. This was not considered treatment related or toxicologically significant. Body weights and gains during lactation were comparable to control. Maternal food consumption during gestation and lactation were comparable to control.

No effects of treatment were seen in the parturition data (number of stillborn pups, number of pups dying between birth and lactation day 4, gestation index, duration of gestation, females completing delivery and mean number of live pups/litter).

The absolute organ weights and organ to body and organ to brain weight ratios of the parental males and females were unremarkable. No treatment effects were evident. There were no treatment related macroscopic or microscopic abnormalities observed in the reproductive study phase. The mean numbers of uterine implantation scars and corpora lutea were comparable between the control and treated groups.

<u>Litter Data</u>
Pup body weights, pup viability indices, and sex ratios were unremarkable. No treatment related effects were evident. There was a slight increase in the number of female pups in the mid dose group at birth. However, in the absence of a similar finding in the high dose, this was not considered a treatment effect. No malformations were seen in stillborn pups or in dead pups found during days 0-4 of lactation in the control or treated groups. There were no macroscopic findings in the pups that were considered related to test material administration.

The No Observed Effect Level for the reproductive study phase was 1000 mg/kg/day.

	The No Observed Effect Level for the overall study was 1000 mg/kg/day.
Remarks	The No Observed Effect Level (NOEL) in this combined 28-day repeated dose oral toxicity, neurotoxicity and reproductive screening study in rats was 1000 mg/kg/day.
Conclusions	This test material did not exhibit any evidence of toxicity when evaluated in a combined 28-day repeated dose oral toxicity, neurotoxicity and reproductive screening study in rats. The NOEL was 1000 mg/kg/day.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 8/9/01

4.3 Genetic Toxicity

Robust Summary #: 25-GenTox-1

Test Substance	
CAS #	67762-72-5
Chemical Name	2,5-Pyrrolidinedione,1-[2-[[2-[[2-[(2-aminoethyl)amino]ethyl]amino]ethyl]amino] ethyl]-, monopolyisobutenyl derivatives
Remarks	This substance is referred to as mono alkenyl succinimide derivative in the HERTG's Test Plan for the Succinimide Dispersant Category. For more information on the chemical, see Section 2.0 "Chemistry of Succinimide Dispersants" in the HERTG's Final Submission for the Succinimide Dispersant Category.
Method	
Method/Guideline followed	OECD Guideline 471
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	Y
Year (Study Performed)	1986
Test System	*Salmonella typhimurium*
Strains Tested	*Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	0.0333, 0.1, 0.3333, 1.0 and 3.333 mg/plate
Metabolic Activation	With and without 400 ul/plate S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats)
Vehicle	Tetrahydrofuran diluted 1:10 with dimethylsulfoxide (DMSO)
Tester strain, activation status, Positive Controls and concentration level	TA98 +S9 2-aminoanthracene 2.0 ug/plate TA98 -S9 2-nitroflourene 10.0 ug/plate TA100 +S9 2-aminoanthracene 2.0 ug/plate TA100 -S9 sodium azide 1.0 ug/plate TA1535 +S9 2-aminoanthracene 2.0 ug/plate TA1535 -S9 sodium azide 1.0 ug/plate TA1537 +S9 perylene 30.0 ug/plate TA1537 -S9 9-aminoacridine 50.0 ug/plate
Vehicle Control	Tetrahydrofuran diluted 1:10 with dimethylsulfoxide (DMSO)
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.
Dose Rangefinding Study	Conducted using tester strain TA100 at dose levels of test material ranging from 0.003 to 3.3 mg/plate without S9.
S9 Optimization Study	Conducted using tester strains TA98 and TA100, and a dose level of test material of 3.3 mg/plate and concentrations of S9 mix ranging from 25 to 400 ul S-9/plate.

Remarks field for test conditions	This study was conducted according to OECD Guideline 471 (1983). Revisions to this Guideline in 1997 suggest the addition of the *E. coli* WP2 uvrA or *S. typhimurium* TA 102 tester strains. Since this study was conducted prior to this revision, these strains were not included. In the main study there were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with five concentrations of test substance, vehicle controls, and a positive control. Three plates/dose group/strain/treatment set were evaluated. 0.1 ml of test material, positive control or vehicle control were added to each plate along with 0.1 ml of tester strain, S9 mix (if needed) and 2.5 ml of top agar. This was overlaid onto the surface of 25 ml minimal bottom agar in a petri dish. Plates were incubated for 48 hours at 37^{o}C. The condition of the bacterial background lawn was evaluated for cytotoxicity and test article precipitate. An Artek 880 Automatic Colony Counter System was used to count all plates of strains TA1535 and 1537 containing >75 revertant colonies and all plates of strains TA98 and 100 unless precluded by compounding factors (precipitate, agar bubbles). A Quebec Darkfield Counter was used for hand counting all other plates. In order for the test material to be considered positive, two consecutive dose levels (or the highest non-toxic dose level) must produce at least twice (2.5 fold for TA1535, 1537 and 1538) the mean number of revertant counts of the negative/solvent control and these consecutive dose levels must demonstrate a dose response relationship.
Results	The test substance was not mutagenic in this assay with or without metabolic activation.
Remarks	No cytotoxicity was observed in the dose rangefinding study with tester strain TA100 with or without metabolic activation. The S9 optimization study was performed using TA98 and TA100 at 3.3 mg/plate and concentrations of S9 mix of 25-400 ul. In the absence of any effect 400 ul S9 mix/plate was used in the mutagenicity study. In the main study the test material was completely miscible with tetrahydrofuran and partially miscible in subsequent dilutions with DMSO, but was not completely miscible with the top agar at ≥0.03 mg/plate. The test material was not cytotoxic to any strain. nor mutagenic to any strain. No reproducible increases in mutation frequency were observed in any tester strain with or without metabolic activation. The tester strains responded to the positive controls as expected.
Conclusions	Under the conditions of this study, the test material was not mutagenic with or without metabolic activation.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 8/7/01

Robust Summary #: 25-GenTox-2

Test Substance	
CAS #	67762-72-5
Chemical Name	2,5-Pyrrolidinedione,1-[2-[[2-[[2-[(2-aminoethyl)amino]ethyl]amino]ethyl]amino] ethyl]-, monopolyisobutenyl derivatives
Remarks	This substance is referred to as mono alkenyl succinimide derivative in the HERTG's Test Plan for the Succinimide Dispersant Category. For more information on the chemical, see Section 2.0 "Chemistry of Succinimide Dispersants" in the HERTG's Final Submission for the Succinimide Dispersant Category.
Method	
Method/Guideline followed	Consistent With OECD Guideline 476
Test Type	Mouse Lymphoma Mutagenicity Screen
GLP (Y/N)	Y
Year (Study Performed)	1986
Test System	L5178Y mouse lymphoma cells Clone 3.7.2C
Culture Preparation and Maintenance	Cell stocks were stored frozen in liquid nitrogen. Cultures were grown in Fisher's medium in a shaker incubator at 125 rpm and 37^{o}C in humidified 5% CO_2 in air and maintained with cell density adjustments at least twice a week for approximately four months. Each time a culture was used it was checked for bacterial or fungal contamination. Prior to use in the assay, cells were treated with methotrexate to reduce the frequency of spontaneously occurring TK-/- cells.
Exposure Method	Dilution
Test Substance Doses/concentration levels	**With metabolic activation: 0.0, 333, 667, 1000, 3330, 6670 ug/ml.** Without metabolic activation Assay A: 0.0, 333, 667, 1000, ug/ml. Without metabolic activation Assay B: 0.0, 500, 1000, 1333, 1667, 2000 ug/ml.
Metabolic Activation	Aroclor 1242/1254 induced rat liver
Vehicle	5% Pluronic F-68 (w/w in distilled water)
Positive Control concentration levels by activation status	With activation: 7,12-dimethylbenzanthracene (DMBA) 5 ug/mL Without activation: ethylmethanesulfonate (EMS) 744 ug/mL
Statistical Analysis	Means and standard deviations were determined. Plates were scored for total number of colonies/plate. Counts were made using an automatic colony counter. Mutation frequency was determined by dividing the average number of colonies in the treated plates by the average number of colonies (x 10^6) in the corresponding vehicle control plates and multiplying by two hundred. By comparing the mutation frequency of the treated plates to that of the control plates, the presence of a significant level of mutagenic activity can be detected.
Test Substance Solubility	Test substance solubility in the vehicle was determined prior to the initiation of the mutagenicity assay.

Toxicity Determination	A preliminary toxicity test with and without S-9 activation was conducted at dose levels ranging from 1 to 5000 ug/ml. The test material was added to each culture tube. After 4 hours the cells were washed and placed into culture for 2 days at 37°C. Test material toxicity was determined by comparing cell population growth at each dose level with that of the solvent controls. Cell population density was determined 24 and 48 hours after the initial exposure to the test material. At 24 hours cell population density was adjusted to 0.3×10^6 Test material toxicity was determined by comparing the total suspension growth of the treated cultures at each dose level with that of the average total suspension growth of the negative (solvent) controls. Cell counts were determined using an electronic cell counter.
Mutagenicity Assay (Remarks field for test conditions)	This study was conducted prior to the development of OECD Test Guideline 476. This study deviates from this guideline in that colony sizing was not performed. This deviation from the guideline was not considered sufficient to invalidate this study. Each test material concentration and the positive controls were tested in singlet. Negative controls were tested in duplicate. Based on the toxicity determination the test material was prepared so that the highest concentration would yield a percent total growth of $\geq$10%. The test material was solubilized and serial dilutions were carried out in order to yield approximately 90% total growth at the lowest dose level. The test material was added to cells, with and without activation, placed on a roller drum at 25 rpm at 37°C for 4 hours. Cells were then washed and placed in suspension cultures at a cell density of 0.3×10^6 cells/ml. In the mutagenicity study there were two treatment sets for each concentration of test substance, with (+S9) and without (-S9) metabolic activation. DMBA (positive control) was tested with activation and EMS (positive control) was tested without activation. The washed cells were replaced on the mixer at 37°C for two days. Cell population adjustments were made at 24 hours to yield a cell population of 0.3×10^6 cells/ml. The cells were then plated in a restrictive media containing trifluorothymidine (TFT) which allows only the $TK^{-/-}$ cells to grow. Cells were also plated in a non-restrictive media that indicated cell viability. Plates were incubated at 37°C in a humidified 5% CO_2 atmosphere for 10-12 days. Following incubation all plates were scored for total number of colonies/plate. The frequency of mutation by dose was determined by comparing the number of colonies in the mutagenicity plates to the number of colonies in the corresponding viability plates. For the study to be acceptable the following criteria must be met: mutation frequency of positive controls with or without activation should be twice that of the solvent control; the negative control spontaneous mutation frequency should be in the range of 10 to $100/10^6$ cells; negative control plating efficiency should be at or above 50%. The test material should be tested to the level of approximately 10% total growth, or the limits of solubility or to a high dose of 10 mg/ml. The following criteria were used as guidelines in judging the significance of test material activity: Positive – the mutation frequency of one or more test material concentrations, with $\geq$10% total growth, is $\geq$ 2 times the average mutation frequency of the negative controls. Negative - none of the dose levels exhibit a 2x increase in mutation frequency over background (solvent control).

Results	The test substance was not mutagenic in this assay with or without metabolic activation.
Remarks	In the mutagenicity assay the test material was diluted with 5% Pluronic F-68 (w/w in distilled water) and tested over a range of concentrations to the limit of solubility. After the two-day expression period, 5 cultures with metabolic activation and 3 and 5 cultures without activation were selected for cloning based on the degree of observed toxicity. Percent total growth ranged from 48% to 89% with activation and 10% to 50% without activation. The positive controls responded appropriately. None of the cultures treated with test material at a range of concentrations up to the limit of solubility, with or without metabolic activation exhibited a mutation frequency that was two times that of the average mutation frequency of the negative controls. Under the conditions of this study the test material was not mutagenic.
Conclusions	The test substance was not mutagenic in this assay with or without metabolic activation.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 8/7/01

Robust Summary #: 25-GenTox-3

Test Substance	
CAS #	84605-20-9
Chemical Name	Amines, polyethylenepoly-, reaction products with succinic anhydride polyisobutenyl derivatives
Remarks	This substance is referred to as Bis alkenyl succinimide derivative in the HERTG's Test Plan for Succinimide Dispersant Category. For more information on the chemical, see Section 2.0 "Chemistry of Succinimide Dispersants" in the HERTG's Final Submission for the Succinimide Dispersant Category.
Method	
Method/Guideline followed	OECD Guideline 474
Test Type	Mammalian Erythrocyte Micronucleus Test
GLP (Y/N)	Y
Year (Study Performed)	1996
Species	Mouse
Strain	Swiss Albino Crl:CD-1 (ICR)BR, 57 days of age at initiation of dosing
Route of administration	Intraperitoneal
Duration of test	Single dose followed by 72-hour evaluation period.
Doses/concentration levels	0, 1250, 2500, 5000 mg/kg
Dose volume	10 ml/kg
Sex	Males and females
Frequency of treatment	Once
Control and treatment groups	Peanut oil vehicle control: 15/sex; Cyclophosphamide positive control: 60 mg/kg (in sterile water), 5/sex; 1250, 2500, 5000 mg/kg: 15/sex.
Statistical methods	Data summarized by sex and dose group/time point. Analysis performed using an analysis of variance, Dunnett's test, and Cochran-Armitage test for linear trend
Dose Rangefinding Studies	Two dose range finding studies were conducted at the following dose levels: 1) 41.2, 412, 4120 mg/kg; 2) 1625, 2750, 3875, 5000 mg/kg; Animals observed for signs of toxicity and mortality.
Remarks field for test conditions	All animals were observed immediately after dosing and periodically throughout the study for toxic signs and/or mortality. Five/sex from each treatment group and vehicle control group were sacrificed for bone marrow sampling 24, 48 and 72 hours post treatment. Positive controls were sampled at 24 hours only. Necropsies were not performed. Bone marrow smears were scored for micronuclei and the polychromatic erythrocyte (PCE) to normochromatic erythrocyte (NCE) cell ratio. 1000 PCEs were scored per animal (Guideline calls for 2000/animal to be evaluated. This difference from the current guideline was not considered sufficient to affect the reliability of the study.) The frequency of micronucleated cells was expressed as percent micronucleated cells based on the total PCEs present in the scored optic field. The frequency of PCEs vs NCEs was determined by scoring the number of each observed in the optic field while scoring at least the first 1000 erythrocytes. If the test article induced neither a statistically significant dose response nor a statistically significant increase at any dose level above

	concurrent vehicle, at any sampling point, it was considered negative.
Results	
Remarks	Based on the data observed in the rangefinding studies the maximum tolerated dose was estimated to be 5000 mg/kg. Dose levels selected for the main study were 1250, 2500 and 5000 mg/kg All vehicle and positive control and 1250 mg/kg/day animals were normal after dosing and remained healthy until the appropriate harvest times. The 5000 mg/kg males and females were slightly hypoactive at 19 and 42 hours post dosing. This finding was also observed at 66 hours in the 5000 mg/kg males only. At 42 and 66 hours post dosing the males at this dose level also exhibited rough hair coats. Females were normal. The 2500 mg/kg males were slightly hypoactive at 42 and 66 hours post dosing. At 66 hours post dosing the males at this dose level also exhibited rough hair coats. Females were normal. Some evidence of bone marrow toxicity was observed as the test material did induce statistically significant decreases in the PCE: NCE ratio in the 2500 mg/kg males and 5000 mg/kg females at the 72 hour evaluation interval and in the 5000 mg/kg males at 48 and 72 hours. A statistically significant increase in micronucleated PCEs was observed in the 5000 mg/kg males at 24 hours. This increase was attributed to the low number of micronucleated PCEs in the concurrent control group. In addition there was no dose response and the value (0.1%) was within the historical control range for the laboratory. No other statistically significant increases in micronucleated polychromatic erythrocytes over the levels observed in the vehicle controls occurred at any of the other harvest times. The positive control induced statistically significant increases in micronucleated PCEs in both sexes compared to the vehicle controls.
Conclusions	The test material was considered negative under the conditions of this study.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 8/8/01

Robust Summary #: 25-GenTox-4

Test Substance	
CAS #	84605-20-9
Chemical Name	Amines, polyethylenepoly-, reaction products with succinic anhydride polyisobutenyl derivatives
Remarks	This substance is referred to as Bis alkenyl succinimide derivative in the HERTG's Test Plan for Succinimide Dispersant Category. For more information on the chemical, see Section 2.0 "Chemistry of Succinimide Dispersants" in the HERTG's Final Submission for the Succinimide Dispersant Category.
Method	
Method/Guideline followed	OECD Guideline 471
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	Y
Year (Study Performed)	1996
Test System	*Salmonella typhimurium* and *Escherichia Coli*
Strains Tested	*Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537; *Escherichia Coli* tester strain WP2*uvr*A
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	Initial assay: *Salmonella* + (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate *Salmonella* - (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate WP2*uvr*A + (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate WP2*uvr*A - (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate Confirmatory assay: *Salmonella* + (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate *Salmonella* - (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate WP2*uvr*A + (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate WP2*uvr*A - (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate
Metabolic Activation	With and without (500 ul of 10% S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats)
Vehicle	Pluronic F127 (25% w/w in ethanol)
Vehicle Control	Pluronic F127 (25% w/w in ethanol)
Tester strain, activation status, Positive Controls and concentration level	TA98 +S9 benzo(a)pyrene 2.5 ug/plate TA98 -S9 2-nitroflourene 1.0 ug/plate TA100 +S9 2-aminoanthracene 2.5 ug/plate TA100 -S9 sodium azide 2.0 ug/plate TA1535 +S9 2-aminoanthracene 2.5 ug/plate TA1535 -S9 sodium azide 2.0 ug/plate TA1537 +S9 2-aminoanthracene 2.5 ug/plate TA1537 -S9 ICR-191 2.0 ug/plate WP2*uvr*A +S9 2-aminoanthracene 25.0 ug/plate WP2*uvr*A –S9 4-nitroquinoline-N-oxide 1.0 ug/plate
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.
Dose Rangefinding Study	Conducted using tester strains TA100 and WP2*uvr*A and ten doses of test material ranging from 10.0 to 10,000 ug/plate, one plate/dose with (10% S9 homogenate/ml of S9 mix) and without metabolic activation.

	Cytotoxicity was evaluated.
S9 Optimization Study	Conducted using tester strains TA98 and TA100, and a non-cytotoxic dose level of test article (10,000 ug/plate) and four concentrations of S9 mix (5, 10, 20 and 80% S9 homogenate/ml of S9 mix). Cytotoxicity was evaluated.
Remarks field for test conditions	In the main study there were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with six concentrations of test substance, vehicle controls, and a positive control. Three plates/dose group/strain/treatment set were evaluated. The results of the initial assay were confirmed in a second independent experiment. 50 ul of test material, positive control or vehicle control were added to each plate along with 100 ul of tester strain, S9 mix (if needed) and 2.0 (with S9) or 2.5 ml (without S9) of top agar. This was overlaid onto the surface of 25 ml minimal bottom agar in a petri dish. Plates were incubated for 48 hours at 37^{o}C. Plates that were not evaluated immediately were held at 5^{o}C until evaluated. The condition of the bacterial background lawn was evaluated for cytotoxicity and test article precipitate. The number of revertant colonies/plate for the vehicle controls and all plates containing test article were counted manually. The number of revertant colonies/plate for the positive controls were counted by automated colony counter.
Results	The test substance was not genotoxic in this assay with or without metabolic activation.
Remarks	In the dose rangefinding study no cytotoxicity was observed with tester strain TA100 or WP2*uvr*A at dose levels up to 10,000 ug/plate with or without metabolic activation. Test article precipitate was observed on plates at 3,330 ug/plate and above with tester strain TA100 and WP2uvrA with and without metabolic activation. Based on these results the dose levels outlined above (page 1, Test Substance Doses, Initial Assay) were selected. The S9 optimization study was performed using TA98 and TA100 with a non-cytotoxic dose of test article, (10,000 ug/plate) and four concentrations of S9 mix (5, 10, 20 and 80% S9 homogenate/ml of S9 mix). In the absence of any effect a 10% S9 mix was used in the mutagenicity study. In the initial assay all data were acceptable and no positive increases in the number of revertants/plate were observed. The confirmatory assays were conducted using the same dose levels. In these confirmatory mutagenicity assays all data were acceptable and no positive increases in the number of revertants/plate were observed with any of the tester strains with or without metabolic activation. However, the vehicle control values for three tester strains (TA98, 1535 and 1537) were higher then routinely expected with metabolic activation. Based on these results these three tester strains were retested for confirmation again. In these confirmatory mutagenicity assays all data were acceptable and no positive increases in the number of revertants/plate were observed. Vehicle control values were within routinely expected values. Based on these results the test material was considered not mutagenic. No cytotoxicity was observed up to 10,000 ug/plate with the

	Salmonella tester strains with and without activation and with WP2*uvr*A with and without activation. Test material participate was observed on plates at ≥500 ug/plate. The positive control for each respective test strain exhibited at least a 3-fold increase (with or without S9) over the mean value of the vehicle control for a given strain, confirming the expected positive control response.
Conclusions	Under the conditions of this study, the test material was not mutagenic.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 8/6/01

4.4 Reproductive/Developmental Toxicity

Robust Summary 25 – Repro/Dev – 1

Test Substance	
CAS #	CAS# 84605-20-9
Chemical Name	Amines, polyethylenepoly-, reaction products with succinic anhydride polyisobutenyl derivatives
Remarks	This substance is referred to as Bis alkenyl succinimide derivative in the HERTG's Test Plan for Succinimide Dispersant Category. For more information on the chemical, see Section 2.0 "Chemistry of Succinimide Dispersants" in the HERTG's Final Submission for the Succinimide Dispersant Category.
Method	
Method/Guideline	OECD Guidelines 421 and 407, EPA Guideline 81-8, 82-7, 83-1
Test Type	Combined four week repeated dose oral toxicity, reproduction and neurotoxicity screen in rats
GLP (Y/N)	Y
Year (Study Performed)	1997
Species	Rat
Strain	Sprague-Dawley CD, 51 days of age at initiation of treatment
Route of administration	Orally by gastric intubation
Duration of test	28 Day toxicity phase: 29 or 30 days Reproduction phase: F0 males: 29 day premating period plus mating and postmating periods (70 days total). F0 female: 29 day premating period through day 4 of lactation (54-68 days total). Neurotoxicity phase: 29 days
Dose levels	28 Day toxicity phase: 0, 100, 500 and 1000 mg/kg/day Reproduction phase: 0, 100, 500 and 1000 mg/kg/day Neurotoxicity phase: 0, 1000 mg/kg/day
Vehicle control	Corn Oil
Dose volume	5 mL/kg
Sex	Males and females
Frequency of treatment	Once/day, 7 days/week
Analytical confirmation of concentration.	Homogeneity, stability and weekly dose concentration confirmation.
Control and treatment groups	28-Day toxicity phase: 6/sex/group in all groups plus 6/sex recovery in control and high dose groups. Reproduction phase: 12/sex/group Neurotoxicity phase: 6/sex/group in the control and high dose plus 6/sex recovery in control and high dose groups.
Post exposure recovery period	28 Day toxicity phase: 2 weeks Neurotoxicity phase: 2 weeks
Mating ratio	One male to one female
Duration of mating period	Up to 9 days with initial male; if positive evidence of mating not present (sperm or copulatory plug) then female paired with a second proven breeder male from the same dose group for up to six additional days.
Statistical methods	Anova, Bartlett's test, Dunnett's, Kruskal-Wallis, Dunn's Ranked Sum, Jonckheere's test; F test, Welch's test, t-test, Fisher's Exact Test with Bonferonni correction.

Dose range finding study	3/sex/group at dose levels of 0, 100, 500 and 1000 mg/kg/day treated for 7 days.
Remarks field for test conditions	*Range-Finding, 28 Day Toxicity, Neurotoxicity and Reproduction Study* Viability and Toxicity: twice daily, at least 4 hours apart. Clinical Observations: pretest and weekly throughout the study. Body Weights: pretest, day 1 of treatment, weekly through termination. F0 males: pretest, weekly during premating, mating and post mating periods and at termination. F0 females: pretest, weekly during premating and mating; gestation days 0, 7, 14 and 20; females with litters weighed on lactation days 0 and 4. Unmated females were weighed weekly. Food Consumption: Pretest and weekly during treatment and recovery periods. F0 males: pretest, weekly throughout the study except during mating. F0 females: pretest, weekly during premating, days 0-7, 7-14 and 14-20 of gestation and days 0-4 of lactation for females with litters. Necropsy: 3/sex/group. Macroscopic Examinations: Macroscopic examinations performed on all animals. 28 Day Toxicity Study Phase Hematology, Clinical Chemistry and Urinalysis: 6/sex/group all groups at termination of treatment, 6/sex from the control and high dose group at completion of the two-week recovery period. **Necropsy: Treatment termination-6/sex/group, macroscopic examination. Recovery termination-6/sex/Control and High Dose, macroscopic examination.** Macroscopic Examinations: Macroscopic examinations performed on all animals. Selected organs weighed for all animals. A range of tissues was preserved for all animals in the control and high dose groups. Microscopic Examinations: Gross lesions and tissue masses were examined microscopically for all animals. Neurotoxicity Study Phase Functional Observational Battery (FOB): 12/sex from the control and high dose group pretest and on days 14 and 29 and 6/sex from the control and high dose on day 42. All animals were tested prior to dosing. Animal groups were blind to the evaluator. The FOB included an evaluation of posture, vocalization and palpebral closure, reactivity to general stimuli, assessment of signs of autonomic function including lacrimation, salivation, fur appearance, deposits around the eyes, arousal level and gait, urination and defecation counts, convulsions, tremors, abnormal movements or behaviors, excessive or repetitive actions, piloerection and exophthalmos, approach response, auditory stimuli, tail pinch response, papillary function, hindlimb extensor strength, grip resistance, landing foot splay, air righting ability and body weight. Necropsy: Treatment termination-6/sex/Control and High Dose, macroscopic examination (Day 30). Recovery termination-6/sex/Control and High Dose macroscopic examination (Day 46). Macroscopic Examinations: All males in the control and high dose were perfused for better fixation of the CNS and peripheral nervous tissues. Brain weight and size recorded. The brain and spinal cord were sampled for neuropathological evaluation. Tissues sampled included: brain (forebrain, center of cerebrum, midbrain, cerebellum, pons, medulla oblongata), spinal cord (at cervical swellings C3-C7 and at lumbar swellings T13-L4), Gasserian ganglion, Lumbar dorsal route ganglion (T13-L4), Lumbar dorsal root fibers (T13-L4), Lumbar ventral root fibers (T13-L4), Cervical dorsal route ganglion (C3-C7),

	Cervical dorsal root fibers (C3-C7), Cervical ventral root fibers (C3-C7), Sciatic nerve (mid thigh region), Sciatic nerve (at sciatic notch), Sural, Tibial, Peroneal nerves, Optic nerve and eyes. Microscopic Examinations: The tissues outlined above and all gross lesions and tissue masses were examined microscopically for all neurotoxicity animals. Reproductive Study Phase Pup/Litter Examinations: Litters observed as soon as possible after delivery for number of live and dead pups and pup abnormalities. Thereafter litters observed twice daily for dead pups and/or obvious irregularities through day 4 of lactation. Litter Size: Number of live and dead pups recorded on days 0 and 4 of lactation. Individual Pup Body Weights: Pup weights recorded on days 0 and 4 of lactation. Pup Sex Distribution: Number of male and female pups in each litter recorded on days 0 and 4 of lactation. Necropsy: F0 males Day 71(2 days after the last litter reached lactation Day 4); F0 females with litters sacrificed on lactation Day 4; Unmated females sacrificed 25 days after completion of mating period; Mated females that did not deliver sacrificed 25 days after completion of mating period; F1 pups were sacrificed on lactation Day 4. Macroscopic Examinations: Macroscopic examinations performed on all F0 animals. F1 pups were examined externally and internally including internal sex verification. Tissues retained for possible skeletal evaluation. Selected organs weighed for all animals. A range of tissues was preserved for all animals in the control and high dose groups. Microscopic Examinations: Select tissues examined microscopically for all control and high dose F0 animals. Gross lesions and tissue masses were examined microscopically for all F0 animals.

Results	*Dose Range-Finding Study* No effects of the test material were observed during the dose range finding study on animal survival, clinical observations, body weight and body weight change, food consumption and feed efficiency or postmortem data. The No Observed Effect Level for the dose range-finding study was 1000 mg/kg/day. ***28 Day Toxicity and Neurotoxicity Study Phases*** All animals survived to their scheduled sacrifice. Clinical observations were unremarkable. Body weight and body weight change data were unremarkable in all groups during treatment and recovery. Food consumption data at 100 and 500 mg/kg/day were comparable to control during treatment and recovery. In the 1000 mg/kg/day group food consumption was significantly higher than control during treatment and continued to be elevated in the females during recovery. Feed efficiency in this group was generally comparable to control; however values were lower than control in the 1000 mg/kg/day males at week 4 and during the first week of recovery in the females. Due to the lack of a consistent trend in these data the observed differences from control were not considered by the Study Director, or by this reviewer, to be of toxicological significance. No effects of treatment were observed in the functional observational battery data of the 1000 mg/kg/day group. Landing foot splay distances in the 1000 mg/kg/day group were slightly shorter than control during treatment and recovery. However these data were similar to pretest values. These values did not change during treatment. Neurological insults are usually associated with a lengthening of this distance. This finding was not considered toxicologically significant or treatment related. No treatment related effects were evident in the hematology, clinical chemistry, urinalysis, organ weight, brain size or macroscopic data of the treated animals in the 28-day toxicity or neurotoxicity study phases. The microscopic examination of gross lesions did not indicate any treatment related microscopic findings in the 28-day toxicity study phase. In the neurotoxicity study phase all tissues were within normal limits when examined by light microscopy. The No Observed Effect Level for the 28-day toxicity and neurotoxicity study phases was 1000 mg/kg/day. *Reproductive Study Phase* *F0 (Parental Generation)* All control and treated animals survived to their scheduled sacrifice. Clinical findings were unremarkable. Mean body weight, body weight change, food consumption and feed efficiency were generally unremarkable during the premating period. In some instances body weight gain and food consumption values were slightly elevated compared to control. These differences from control were not considered toxicologically significant. Mean male body weights of the treated animals were generally considered comparable to control during mating and post mating. Some mid and high dose animals exhibited slight weight loss during the last two weeks of the study. The cause of this finding was unclear. However the lack of any body weight effects during the first eight weeks of study and the lack of any effect on body weight gain suggests that this finding was not treatment related. Postmating period food consumption for the treated males was higher than control (frequently statistically significantly) over the postmating period. This increase in food consumption

	was not considered an adverse effect or toxicologically significant. Feed efficiency was reduced in the 500 and 1000 mg/kg/day males during the last two weeks of study. These decreases coincided with the reduced body weights observed in some animals in these groups. Female mating indices for the treated groups were comparable to control. Male mating indices were comparable to control (100%) at 100 (100%) and 500 (100%) mg/kg/day and slightly lower than control at 1000 mg/kg/day (83.3%, 10/12). The lower value in the high dose group was not statistically different from control and was within the range of recent historical control data for the laboratory. It was not considered treatment related. Mean maternal body weights during gestation were unaffected by treatment. Body weight gain over days 7-14 of gestation in the mid and high dose groups was significantly higher than control. This was not considered treatment related or toxicologically significant. Body weights and gains during lactation were comparable to control. Maternal food consumption during gestation and lactation were comparable to control. No effects of treatment were seen in the parturition data (number of stillborn pups, number of pups dying between birth and lactation day 4, gestation index, duration of gestation, females completing delivery and mean number of live pups/litter). The absolute organ weights and organ to body and organ to brain weight ratios of the parental males and females were unremarkable. No treatment effects were evident. There were no treatment related macroscopic or microscopic abnormalities observed in the reproductive study phase. The mean numbers of uterine implantation scars and corpora lutea were comparable between the control and treated groups. <u>Litter Data</u> Pup body weights, pup viability indices, and sex ratios were unremarkable. No treatment related effects were evident. There was a slight increase in the number of female pups in the mid dose group at birth. However, in the absence of a similar finding in the high dose, this was not considered a treatment effect. No malformations were seen in stillborn pups or in dead pups found during days 0-4 of lactation in the control or treated groups. There were no macroscopic findings in the pups that were considered related to test material administration. The No Observed Effect Level for the reproductive study phase was 1000 mg/kg/day. The No Observed Effect Level for the overall study was 1000 mg/kg/day.
Remarks	The No Observed Effect Level (NOEL) in this combined 28-day repeated dose oral toxicity, neurotoxicity and reproductive screening study in rats was 1000 mg/kg/day.

Conclusions	This test material did not exhibit any evidence of toxicity when evaluated in a combined 28-day repeated dose oral toxicity, neurotoxicity and reproductive screening study in rats. The NOEL was 1000 mg/kg/day.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 8/9/01